Original Article

Simultaneous estimation of metoprolol succinate and chlorthalidone in pharmaceutical solid dosage form by using a developed and validated reverse phase high performance liquid chromatographic technique

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Abstract

The separation of both the drugs was achieved on Inertsil ODS 3 column (100 × 4.6 mm, 5 µm) column using a mobile phase of diammonium hydrogen phosphate buffer solution (at pH 5.5): Methanol (70:30 v/v). The flow rate was 1.0 ml/min and detection was done at 254 nm. The retention time for metoprolol succinate was 6.91 min and chlorthalidone was 9.94 min. Metoprolol succinate and chlorthalidone showed a linear response in the concentration range of 50-300 µg/ml and 12.5-75 µg/ml, respectively. The correlation coefficients for metoprolol succinate and chlorthalidone were 0.9999 and 0.9998, respectively. The percentage recoveries obtained for metoprolol succinate and chlorthalidone ranges from 98.9 to 100.6% and 98.5to 99.2%, respectively. The results of analysis have been validated as per International Conference on Harmonization (ICH) guidelines. Validation results indicated that method shows satisfactory linearity, accuracy, precision, and ruggedness. The extremely low flow rate, simple mobile phase composition makes this method cost effective, rapid, and nontedious and can also be successfully employed for simultaneous estimation of both drugs in commercial products.

Key Words: Chlorthalidone, high performance liquid chromatograph, metoprolol succinate

INTRODUCTION

Metoprolol succinate [Figure 1] is chemically, 1-(iso-propylamino)-3-[4′(2-methoxyethyl) phenoxy]-2-propanol. Metoprolol succinate is a selective β1 receptor blocker which is used in several cardiovascular diseases, especially hypertension. The literature survey reveals that metoprolol succinate was analyzed by the spectrophotometric, high performance thin layer chromatography (HPTLC), and reverse phase high performance liquid chromatography (RP-HPLC) methods.[1-8] Chlorthalidone [Figure 2] is chemically (RS)-2-chloro-5-(1-hydroxy-3-oxo-2,3 dihydro-1H-isoindol-1-yl)-benzene-1-sulfonamide. It is used as thiazide diuretic drug that has antihypertensive action. Literature survey reveals bioanalytical methods by liquid chromatography-mass spectrometry (LC-MS) for detection of chlorthalidone in human serum and blood, few spectrophotometric methods, and HPLC methods for the quantitative estimation of chlorthalidone in bulk and pharmaceutical formulations.[9-12] Currently, most commonly prescribed medicines for cardiovascular diseases are statins and diuretics. The present drug combination has promising effect to control hypertension and other heart diseases. Literature review suggests no analytical methods reported for simultaneous analysis in combined form, and hence the following work was carried out.

Access this article online

Quick Response Code:

Website: www.scholarsjournal.in

DOI: 10.4103/2249-5975.119801

MATERIALS AND METHODS

Active Pharmaceutical Ingredient (API) Reference standards of metoprolol succinate and chlorthalidone were received from Torrent Pharmaceuticals Ltd., Ahmedabad, Gujarat. Vinicor D tablets were obtained from IPCA Laboratories Ltd., Gujarat. Dose of metoprolol succinate was 50 mg and chlorthalidone was 12.5 mg in Vinicor D tablets. Acetonitrile (HPLC grade) used was purchased from Merck (India) Ltd., Mumbai. Orthophosphoric

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acid and sodium perchlorate were purchased from Spectrochem, India. RP-HPLC was performed using Shimadzu HPLC system (LC 2010CHT, Shimadzu Corporation, Japan) equipped with quaternary pump, auto injector, column oven, and photodiode array (PDA) detector.

Chromatographic Condition

Column: Inerstil ODS 3 (4.6 × 100 mm), 5 µm.
Detector: 254 nm
Injection volume: 20 µL
Flow rate: 1.0 mL/min
Temperature: 30°C
Run time: 14 min
Mobile phase: Buffer (at pH 5.5):methanol (70:30)
Diluent: Methanol as diluent 1 and mobile phase as diluent 2

Experimental Work

Buffer Preparation

Accurately weighed 1.3 g diammonium hydrogen phosphate was dissolved into 1,000 mL milli-q water and 10 ml triethylamine was added to this buffer solution, than pH was adjusted to 5.5 with orthophosphoric acid.

Preparation of Standard Solution

Approximately 100 mg of metoprolol succinate and 25 mg of chlorthalidone reference standards were accurately weighed and transferred to a 200 ml volumetric flask. The weighed sample was dissolved in methanol and sonicated for 10 min. The weighed sample was made up to volume with methanol to produce a standard stock solution. Aliquots from the standard stock solutions were appropriately diluted to 25 ml with mobile phase to obtain final standard concentration of metoprolol succinate (200 ppm) and chlorthalidone (50 ppm).

Preparation of Test Solution

Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 500 mg of metoprolol succinate and 62.5 mg of chlorthalidone was transferred to a 500 ml volumetric flask. A few milliliters of methanol were added to above flask and flask was sonicated for 30 min. The solution was made up to mark with same diluent. Appropriate volume of aliquot was transferred to 25 ml volumetric flask and volume was made up to mark with diluent to obtain final standard concentration of metoprolol succinate (200 ppm) and chlorthalidone (50 ppm). The test solution was filtered through 0.45 µm (Polyvinylidine fluoride (PVDF) Millipore Filter) and analyzed by using HPLC.

Method Optimization

The detection wavelength of 254 nm was chosen in order to achieve a good sensitivity for quantitative determination of metoprolol succinate and chlorthalidone in solid dosage form. The retention time for metoprolol succinate was 6.9 min and chlorthalidone was 9.9 min. The isocratic program throughout HPLC method was adopted to analyze both components in a single run as shown in [Figure 3].

Method Validation

Validation was carried out with respect to various parameters, as required under International Conference on Harmonization (ICH) guideline Q2 (R1). The developed method validated with respect to parameters such as linearity, precision, accuracy, specificity, ruggedness, robustness, and solution stability.[13]

System Suitability and System Precision

The results of system suitability and system precision are presented in Table 1.

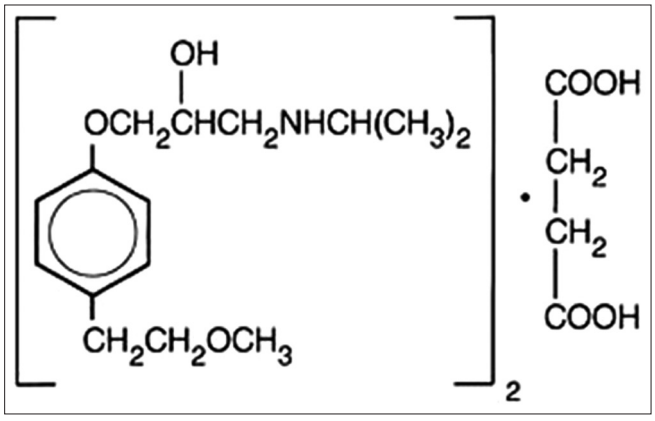

Figure 1: Structure of metoprolol succinate

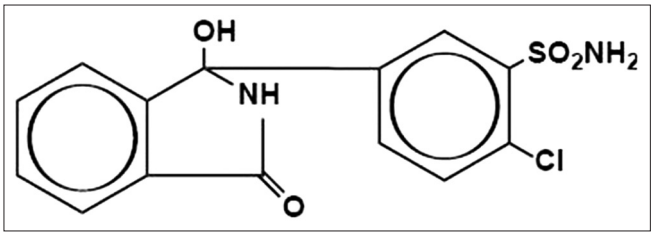

Figure 2: Structure of chlorthalidone

Table 1: System suitability results

Drug	Retention time	Theoretical plates	Asymmetry	Resolution
Metoprolol succinate	6.9±0.0039	9546	1.02	-
Chlorthalidone	9.9±0.0058	5225	1.03	4

Linearity

Appropriate aliquots of metoprolol succinate and chlorthalidone standard stock solutions were taken in different 25 ml volumetric flasks. The volume was made up to mark with mobile phase to yield solutions in final concentration range of 50-300 µg/mL for metoprolol succinate and 12.5-75 µg/mL for chlorthalidone. The solutions were analyzed by using HPLC. Calibration curve for both the drugs are shown in Figures 4 and 5. The results of linearity are presented in Table 2.

Precision

The method precision was done by preparing six different sample preparations by one analyst. The results are presented in Table 3. The results obtained were within 2% relative standard deviation (RSD).

Ruggedness

Ruggedness test was determined between different analyst, instrument, and column. The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method. The results are presented in Table 3.

Accuracy

The agreement between the theoretical added sample amount to the placebo and practically achieved sample amount from placebo has been employed for the determination of accuracy of analytical method. It was achieved at three different levels 50, 100, and 150% of the target concentration in triplicate. The results are presented in Table 4.

Solution Stability

The standard and sample solutions were found stable up to 24 h at room temperature.

Robustness

Robustness of the method was carried out by deliberately made small changes in the flow rate, pH, and organic phase ratio and column oven temperature. Results are presented in Table 5.

Limit of Detection and Limit of Quantitation

LOD is defined as the lowest concentration of an analyte that can be reliably differentiated from background levels. LOQ of an individual analytical procedure is the lowest amount of analyte that can be

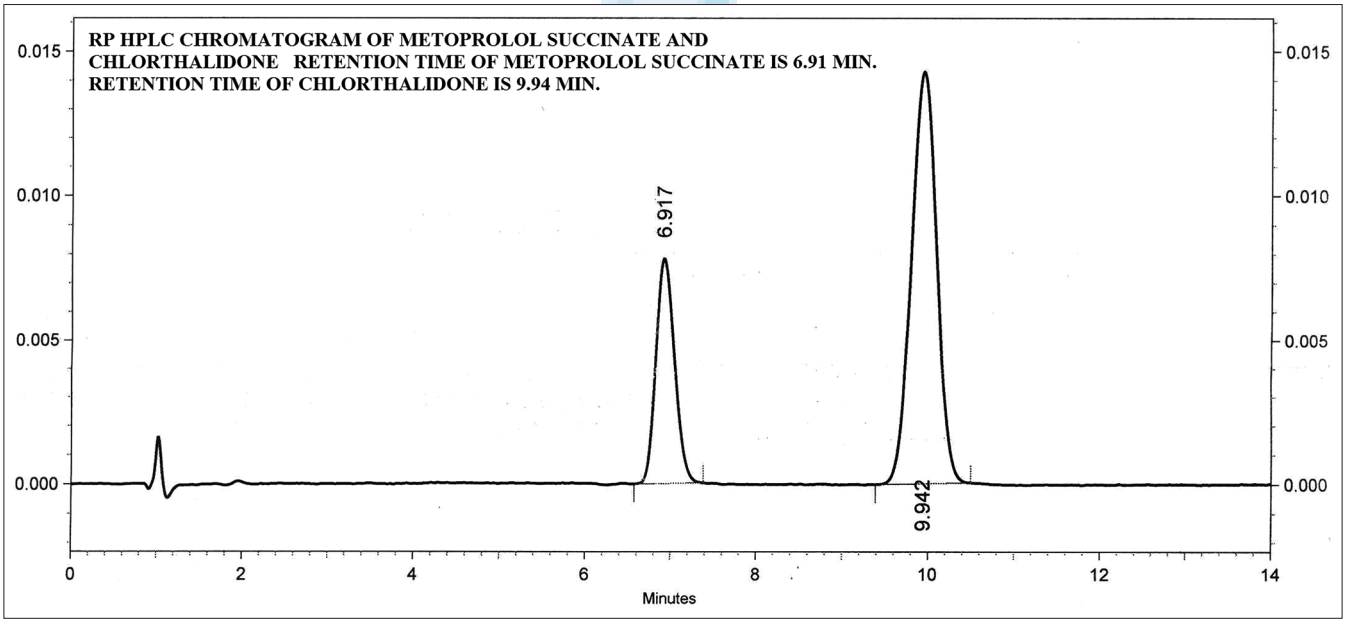

Figure 3: HPLC chromatogram of metoprolol succinate and chlorthalidone

Table 2: Linearity data of metoprolol succinate and chlorthalidone

Linearity range (%)	Stock solution used for linearity (ml)	Diluted to volume (ml)	Final concentration of metoprolol (ppm)	Metoprolol area	Final concentration of chlorthalidone (ppm)	Chlorthalidone area
25	2.50	25	50	29,851	12.5	73,091
50	5.00	25	100	59,702	25	146,182
75	7.50	25	150	89,552	37.5	219,892
100	10.0	25	200	119,403	50	292,364
125	12.5	25	250	149,254	62.5	365,621
150	15.0	25	300	181,105	75	445,221

quantitatively determined with suitable precision and accuracy. LOD and LOQ of metoprolol succinate and chlorthalidone is calculated based on the standard deviation of response (SD) and slope of calibration curve using formula:

$LOD = \sigma/S \times 3.3$
$LOQ = \sigma/S \times 10$

Where, σ is Standard deviation of response and S is slope of calibration curve.

The results of LOD and LOQ are mentioned in Table 6.

RESULT AND DISCUSSIONS

The values of RSD are satisfactorily low and recovery was close to 100% which indicated accuracy and reproducibility of methods.

Table 3: Precision and ruggedness data for metoprolol succinate and chlorthalidone

Parameters	Metoprolol succinate		Chlorthalidone	
	% Assay, mean (*n*=6)	% RSD	% Assay, mean (*n*=6)	% RSD
Method precision	100.4	0.1	100.6	0.6
Ruggedness	100.6	0.3	100.3	1.0

RSD: Relative standard deviation

Table 4: Accuracy data of metoprolol succinate and chlorthalidone

Levels %	Metoprolol succinate		Chlorthalidone	
	% Assay, mean (*n*=3)	% RSD	% Assay, mean (*n*=3)	% RSD
50	98.9	0.9	98.5	0.2
100	99.9	0.1	99.2	0.7
150	100.6	0.5	98.7	0.4

RSD: Relative standard deviation

CONCLUSION

Thus, proposed method was found to be simple, accurate, and precise for routine analysis of metoprolol succinate and chlorthalidone in pharmaceutical solid dosage form by simultaneously adopting this validated method.

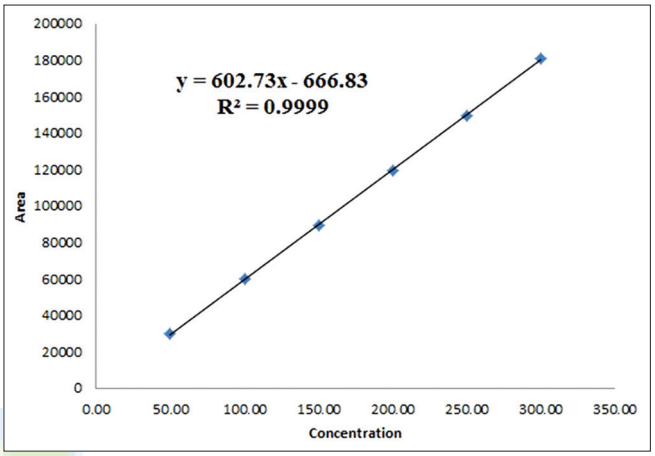

Figure 4: Calibration curve of metoprolol succinate

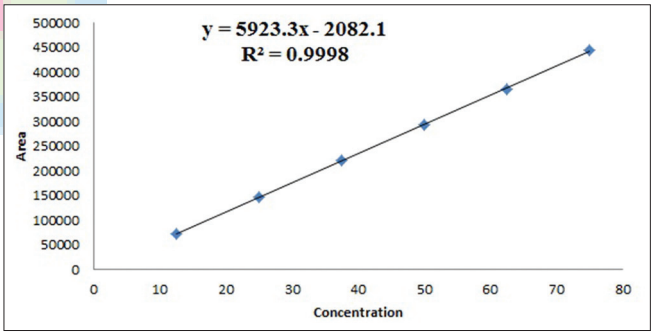

Figure 5: Calibration curve of chlorthalidone

Table 5: Robustness data for metoprolol succinate and chlorthalidone

Changing factor	Robustness study		
	Level	Metoprolol succinate (*n*=6); mean % assay (% RSD)	Chlorthalidone (*n*=6); mean % assay (% RSD)
Column temperature	25°C	99.9 (0.1)	99.5 (0.4)
	35°C	99.9 (0.1)	99.6 (0.4)
Flow rate	1.1 ml/min	99.8 (0.2)	99.2 (0.8)
	0.9 ml/min	99.3 (0.7)	99.8 (0.2)
Organic mixture ratio to buffer (change in organic mixture composition of mobile phase up to 2%)	Buffer: Methanol (72:28)	99.8 (0.2)	99.6 (0.4)
	Buffer: Methanol (68:32)	99.5 (0.5)	99.8 (0.2)
pH of buffer used in mobile phase	pH 5.6 buffer	99.2 (0.8)	99.9 (0.1)
	pH 5.4 buffer	99.5 (0.5)	99.5 (0.5)

RSD: Relative standard deviation

Table 6: Summary of validation parameters

Parameters of validation	Acceptance criteria	Metoprolol succinate	Chlorthalidone
Linearity	Covers the entire range	50-300 µg/ml	12.5-75 µg/ml
Correlation coefficient	Correlation coefficient r^2 >0.9999	0.9999	0.9998
LOD	S/N> 2 or 3	1.0 µg/ml	0.25 µg/ml
LOQ	S/N>10	3 µg/ml	0.75 µg/ml
Precision	RSD< 2%	0.1%	0.6%
Ruggedness	RSD< 2%	0.3%	1.0%
Accuracy	Recovery: 98-102%	99.3-100.4%	99.2-100.4%
Specificity	No interference of blank	Complies	Complies
Solution stability	>12 h	Stable for 24 h; %RSD=0.9	Stable for 24 h; %RSD=0.6
Robustness	RSD NMT 2% in given condition	Complies	Complies

LOD: Limit of detection, LOQ: Limit of quantitation, RSD: Relative standard deviation, S/N: Signal to noise ratio, NMT: Not more than

REFERENCES

1. Phale MD, Hamrapurkar PD. A validated and simplified RP-HPLC of metoprolol succinate from bulk drugs. Asian J Res Chem2009;2:119-22.
2. Jain PS, Patel MK, Bari SB, Surana SJ. Development and validation of HPTLC method for simultaneous estimation of amlodipine besylate and metoprolol succinate in bulk and tablets. Indian J Pharm Sci 2012;74:152-6.
3. Kulkarni MN, Kshirsagar RV, Sakarkar DM. Development and validation of spectrophotometric determination of metoprolol succinate. Int J ChemTech Res 2009;1:1273-7.
4. Durga KB, Mounika IN, Shajhan Sk, Rao SN, Reddy SC, Subramanyam M, *et al*. RP-HPLC method for estimation of metoprolol in bulk drug. Int Sci InnovDiscov 2011;1:151-7.
5. Raval HV, Patel DM, Patel CN. Estimation of metoprolol tartrate and chlorthalidone in combined dosage forms by UV-spectrophotmetric methods. Res J Pharm Technol 2011;4:1132-4.
6. Available from: http://www.drugs.com/mtm/metoprolol.html [Last accessed on 2012 Feb 26].
7. Yilmaz B. Determination of metoprolol in pharmaceutical preparations by zero-, first-, second- and third-order derivative spectrophotometric method. Int J PharmBiosci 2010;1:1-15.
8. Kalisetty S, Reddy TS, Reddy MA, Rao DV, Raju MS, Vyas K, *et al*. Ultra performance liquid chromatographic method development and validation for quantification of impurities and degradation products in the metoprolol succinate er tablets. Int J Pharm Biol Sci 2012;2:247-55.
9. Available from: http://www.drugs.com/mtm/Chlorthalidone.html [Last accessed on 2012 March 29].
10. Mhaske RA, Garole DJ, Mhaske AA, Sahasrabuddhe S. RP-HPLC method for simultaneous determination of amlodipine besylate, valsartan, telmisartan, hydrochlorthiazide, and chlorthalidone: Application to commercially available drug products. Int J Pharm Sci Res2012;3:141-9.
11. Akiful HM, Nivedita G, Prasant KK, Hasan AS, Diwan PV. Simultaneous estimation of atenolol and chlorthalidone in tablet dosage form using UV-spectroscopy. IOSR J Pharm Biol Sci 2012;1:20-3.
12. Khuroo A, Mishra S, Singh O, Saxena S, Monif T. Simultaneous determination of atenolol and chlorthalidone by LC-MS-MS in human plasmaChromatographia 2008;68:721-9.
13. ICH Expert Working Group. The European Agency for the evaluation of medical products Text on validation of analytical procedures. ICH Harmonized Tripartite Guideline ICH-Q2B, Note for guideline on validation of analytical procedure: Methodology, 1996. GPMP/ICH/254/1-18 p. 1-18.

How to cite this article: Sheth, A, Patel CN, Ramlingam B, Shah N. Simultaneous estimation of metoprolol succinate and chlorthalidone in pharmaceutical solid dosage form by using a developed and validated reverse phase high performance liquid chromatographic technique. Scho Res J 2012;2:17-21.

Source of Support: Nil, **Conflict of Interest:** None declared.